Detection of Radial Glial Cell Marker-2 in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
1X Citrate Buffer
DAB Chromagen
Hematoxylin

Blocking Solution: Dakocytomation Protein Block Serum-Free Ready-To-Use
Dakocytomation Corporation
Carpinteria CA 93013
www.dako.com
1-800-235-5763
Code No. X0909

Avidin / Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # SP-2001

Primary Antibody: Mouse Anti-Radial Glial Cell Marker-2 Monoclonal Antibody
Millipore
Billerica, Massachusetts 01821
www.millipore.com
1-800-645-5476
Catalog # MAB5740

Negative Control Serum: Purified Mouse IgM Isotype Control Serum
BD Biosciences
San Jose, CA 95131
www.bdbiosciences.com
1-877-232-8995
Catalog # 550340

Secondary Antibody: Biotinylated Goat Anti-Mouse IgM
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # BA-2020

Label Complex: R.T.U. Vectastain Elite ABC Reagent
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-7100

Staining Procedure

Positive Control Tissue: Embryonic brain
Stain Localization: Cytoplasmic

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Decloaker
   Add 500 ml of distilled water to the pan inside the decloaker.
   Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer
   (Insert blank slides into any empty slots in the rack to ensure even heating of slides)
   Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* _____
   Depressurize for 10 minutes.
   Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides*_____
   Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse slides in 2 changes of 1X Wash Buffer for 5 minutes each.

6. Block with the Dako Protein Blocking Reagent for 10 minutes at room temperature.
   Lot #_____ Exp Date_____

   DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

7. Avidin / Biotin Blocking Kit
   Lot #_____ Exp. Date_____ New Kit: yes / no
   Apply avidin block for 15 minutes at room temperature.
   Quick rinse in 1X Wash Buffer.
   Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SECTIONS WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY. ONLY WIPE EXCESS BLOCK.

8. Apply primary antibody at 1:25 dilution. Incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date _____

   For negative control slides, dilute the protein concentration of the mouse IgM serum to match that of the primary antibody, if necessary. Make a 1:25 dilution from this normalized serum, and apply to the slides. Incubate for 1 hour at room temperature.
   Lot #_____ Date Reconstituted_____

9. Rinse slides in 2 changes of 1X Wash Buffer for 5 minutes each.

10. Apply the goat anti-mouse IgM secondary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.
    Lot #_____ Date Reconstituted_____

11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

12. Apply the Vectastain R.T.U Elite Label and incubate for 30 minutes at room temperature.
    Exp. Date_____ New Kit: yes / no

13. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.

14. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature.
    (Add 1 drop of DAB per ml of substrate)
    Lot #_____ Exp. Date_____ New Kit: yes / no

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with Harris Hematoxylin for 20 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X Wash Buffer until the tissues turn blue.

19. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip